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## Different Sensors Used for Biochemical Detection

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**ABSTRACT:** Biochemical process measurement shows great importance in our daily life. Mainly this paper describes to realize an efficient and proper working sensor to detect the biochemical process. Here we discuss about the design and working process of different types of sensors. First type is microfluidic chip sensor which is an integration of antimony (Sb)–bismuth (Bi) thin-film thermopile heat detection element. It is highly sensitive, inexpensive and easy fabrication. The device measure the dynamic temperature changes which occur at mixing of glycerol and water. Another sensor is a polysilicon wire based biosensor for the detection of glucose and matrix metalloproteinase (MMP) extracted from cancer cells. These are only for the specific sample testing. The current flows through the poly-Si wire by applying some material. Third detector is Biochemically sensitive field-effect sensors. Sensor works based on metal gate complementary metal-oxide semiconductor technology. They measure the current flowing through it by applying some biochemical materials. In this paper, we compare these sensors and study about the design and working. Finally we choose one of the sensor with high sensitive, inexpensive, easy to use.

**KEYWORDS:** Thermoelectric, thermopile, FIB, CAFM.

### I. INTRODUCTION

Biochemical analysis techniques consists a set of methods, assays, and procedures. These techniques are useful to analyse the substances found in living organisms and the chemical reactions involved in their life processes. The most of these techniques are reserved for specialty research and diagnostic laboratories. Although simplified sets of these techniques are used in some other areas such as common events as testing for illegal drug abuse in competitive athletic events and monitoring of blood sugar by diabetic patients. To perform an analysis of a biomolecule in a system, we needs to design a strategy to detect that biomolecule, isolate it in pure form from among thousands of molecules from a biological sample, characterize it, and analyse its function. The first biomolecules are the small building blocks of larger and more complex macromolecules, the amino acids of proteins, the bases of nucleic acids and sugar monomers of complex carbohydrates.

Biochemical detection- is the science and technology of detecting biochemicals and their concentration. Biochemicals involves chemical processes within and relating to living organisms. Chemical reaction is the process of changing one or more chemicals. In our surrounding living organisms such as animals, plants, fungi, protists, bacteria etc. From these organisms we collect some cells, and which are then tested by this detection process. From the result analysis we can observe some fluctuations or some disorder in the output if the cell is damaged. That means the reactions are not proper. This way we can detect early, about the tested samples misbehaviour

Various sensing techniques used for detection of bio chemicals are discussed here. The main purpose of biochemical detection is to provide early detection of prostate cancer, Plant Disease Detection, to Detect Early Alzheimer's Disease, Early detection of breast cancer, animals influenza etc.

## II. LITERATURE SURVEY

### 1) MICROFLUIDIC CHIP SENSOR

#### A. Thermopile

[1]Thin-film thermopile consists no of thermocouples. Each thermocouples is the combination of two metal elements. Here used elements are with antimony (Sb) and bismuth (Bi) metals. Thermal evaporation used for making thermopile. Sb and Bi metals have higher Seebeck coefficient values 47 and  $-72 \mu\text{V K}^{-1}$ , respectively. Seebeck coefficient of thermopile is  $7.14 \mu\text{V (mK)}^{-1}$ . First layer is bi layer and above which a complementary layer, then sb layer. A metal mask also placed for providing leads. Which are connectors to the voltmeter. This sensing material have two junctions which are measuring and reference junctions. See in (Fig. 1).

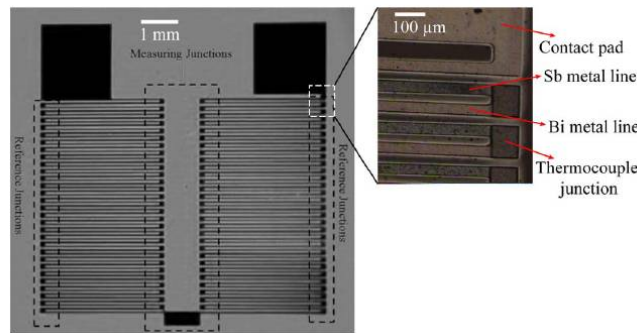


Fig. 1. Antimony (Sb)-bismuth (Bi) thin-film thermopile sensor.

#### B. Microfluidic chip sensor (MCS)

This main element is microfluidic device. Main process involved to yield the microfluidic chip sensor(MCS) is the integration of thermopile sensor in to the channel wall of the device. Microfluidic device is a sandwich of three layers of dual side adhesive tape (kapton.com) between a microscope glass slide and a microscope glass coverslip. The mid layer tape is cut to a pattern for the formation of channel, then device formed by sandwiching process. The device have two

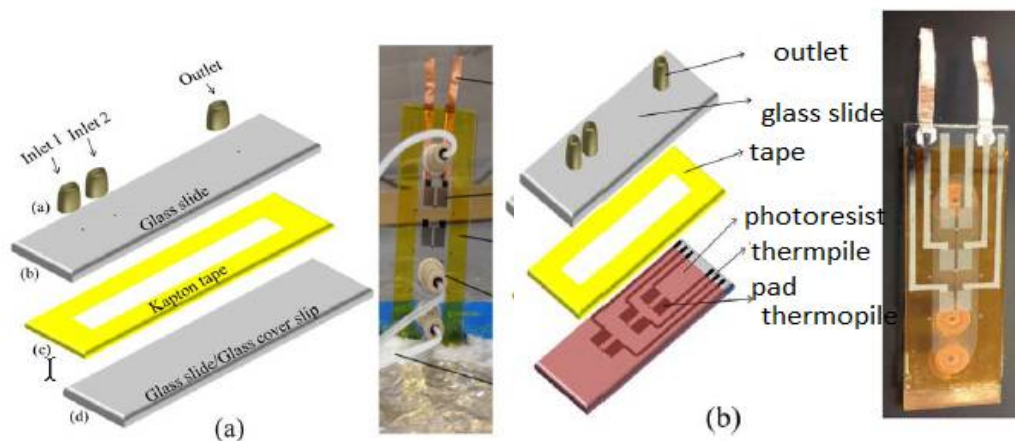


Fig. 2. (a) MCS-1 configuration showing the fabrication of microfluidic device with thermopiles outside the fluidic channel, Fig 2 (b) Schematic of the MCS-2 configuration with a SU-8 layer on thermopiles facing inside of the channel. Pictures next to the schematics in A and B are the fabricated MCS configurations

inlets and one out let for fluid flow. For this we have to drill holes onto the glass slide. Thermopile fabrication procedure described in detail in [9]. Two configurations of the microfluidic chip sensors (MCS-1, MCS-2) were fabricated, tested and evaluated. MCS-1 was fabricated with the glass coverslip containing thermopile sensor facing

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outside of the channel (Fig. 2a). The thermopiles are protected by attaching a 25 μm thick tape to prevent damage due to handling. MCS-2 was fabricated with the coverslip containing thermopile sensor facing the channel (Fig. 2b). The chip sensor have two configurations one is MCS 1, second one is MCS 2. In MCS 1 glass coverslip containing thermopile sensor facing outside of the channel (Fig. 2a). The thermopiles are protected by attaching a 25 μm thick tape. To prevent damage due to handling and in MCS 2 fabricated with the coverslip containing thermopile sensor facing the channel (Fig. 2b). Thermopiles protected by a 3 μm thick SU-8 photoresist layer to protect from the fluid flow.

Thermal resistance (R) for the reaction generated by flow in the microfluidic channel to the thermopile. [4]

$$R = \frac{L}{k \cdot A}$$

where: *L*- thickness of the layer (m),  
*k*-thermal conductivity (W m<sup>-1</sup>K<sup>-1</sup>),  
and *A*-area of the layer (m<sup>2</sup>)

The thermal resistance in the MCS-1 is due to the thermal resistance of the glass coverslip, which is 6.07 KW<sup>-1</sup> and thermal resistance in the MCS-2 only the 3 μm thick SU-8 layer which is 0.833 KW<sup>-1</sup>.

### C. Experimental setup

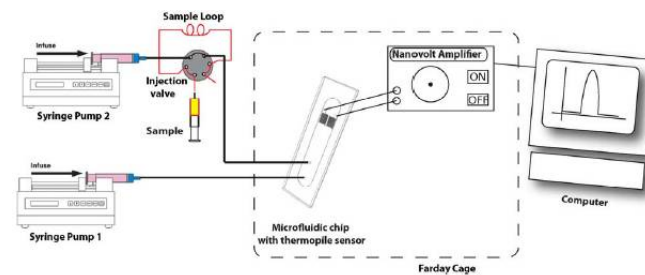


Fig. 3. Schematic of the experimental setup.

Fig. 3 shows working setup. Consists of two syringe pumps, which are used for continuous flow of water into the inlets. Mixing happens only at the interface as viscosity dominates compared to inertia. An injection valve with sample loop is used to inject the sample into the inlet 2 flow for generating a chemical reaction. The inlet 2 flow is hydrodynamically focused into the MCS. This results in a hydrodynamically focused flow [10]. The reaction is generated over the measuring junctions of the thermopile, which generates a proportional voltage. This voltage is detected by a nanovoltmeter and is recorded in a computer.

### 2) POLYSILICON WIRE SENSOR

[2] It proposed a polysilicon (poly-Si) wire is the sensing area Fig 4. It helps for the detection of glucose and matrix metalloproteinase (MMP) extracted from cancer cells. A focus-ion-beam (FIB) processed capillary atomic-force-microscopy (C-AFM) tip is used to help for transferring trace amount of glucose and MMP solutions onto the exact position of the surface of the poly-Si wire sensor.

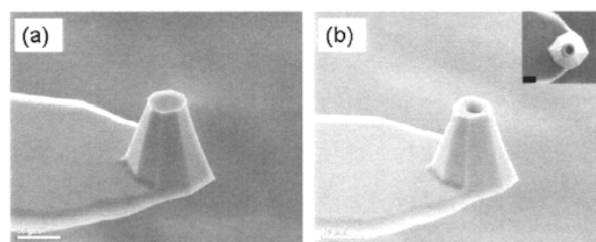


Fig4 AFM tip (a) before and (b)

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Glucose solution with different concentrations and MMPs extracted from humans lung adenocarcinoma cells are then determined from the current flowing through the poly-Si wire. Due to different amount of hydrogen ions bounded to the poly-Si wire surface when different glucose solutions or MMPs were dropped on the poly-Si wire surface, charge accumulation occur in the poly-Si wire and causing different current levels been measured. That means sensitivity is less. Wire sensitivity increased by the use of CAFM tip for solution transfer. This method is effective only for glucose and MMP detection.

In recent years, this technology have more attention because of its small size and high sensitivity[5]-[7]. Silicon nanowire has been used for glucose detection as well as for cancer detection. Coating of an enzyme or oxidase layer on top of the silicon nanowire surface is one of the advantage. It can improve the detection sensitivity or increase the ion selectivity. One of the most difficulty is, when using nanowire for biochemical sensing is to put the substance to be tested to the exact position onto the surface of the silicon nanowire. so here applying a method to carry and drop the solution to be tested to the proper position on the poly-Si wire surface by using an FIB-processed C-AFM tip. Both the glucose solution with different concentrations as well as the MMPs extracted from humans lung adenocarcinoma cells are detected by using the poly-Si wire sensor. Noticeable fact that tumor metastasis is a complicated process that involves coordination of matrix disintegration, cell detachment, cell-cell junction disassembly, and cell migration. Degradation of basement membranes and extracellular matrix (ECM) is required for tumor cell invasion and metastasis. MMPs play an important role in the metastasis of the cancer cells[8] and their expression possesses the potential to directly enhance the growth and spreading of cells. Therefore, this work, we deliberately constructed a microenvironment in which the migration and invasion of cancer cells could be induced and the MMPs extracted from the tumor cells can be test.

In experiment section, a cylindrical well dig into a blunt Si AFM tip contains a flat surface by using FIB milling process. Figure.4 shows the picture of the FIB-processed C-AFM tip. P-type silicon wafer was used as the substrate. After standard RCA clean, a 12 nm-thick thermal oxide was grown at 900°C. Following that, a phosphorous-doped polysilicon layer with a thickness of 80 nm was deposited at 620°C. an e-beam writer is used to define the pattern of the poly-Si wire. An ethanol solution of 3-aminopropyltriethoxysilane (APTES) was then carried and transferred to a few nm above the poly-Si wire by the FIB-processed C-AFM tip. By applying a -5V bias between the tip and the substrate, the repulsive force between the tip and the APTES solution would force the solution to spray onto poly-Si wire surface. The APTES layer was then cured at 120°C for 5 min on a hot plate, which can increase the adhesion of glucose and MMPs with the poly-Si wire surface as well as the sensitivity of the sensor. For glucose detection, an additional glucose oxidase (GOD) layer prepared by dissolving 30 mg GOD (EC.1.1.3.4, Sigma) into a phosphate buffer solution and mixed with 20 $\mu$ L  $\beta$ -D glucose solution has to be deposited onto the poly-Si wire surface. For cancer cell detection, H1299 and A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal buffer solution (FBS) supplemented with penicillin (50 units/ml) and streptomycin (50  $\mu$ g/ml). All cultures were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> mixed with 95% air. The matrix gel was prepared by 24 well culture plates added with 100  $\mu$ L 5.7 x DMEM, 50  $\mu$ L 2.5% NaHCO<sub>3</sub>, 100  $\mu$ L 0.1 M 4-(2-hydroxyethyl)-1piperazineethanesulfonic acid, 10  $\mu$ L 0.17 M CaCl<sub>2</sub>, 15  $\mu$ L and 1 N NaOH. The tumor cell lines would be invasion the matrix gel and secreted MMPs in the 24 well culture plates. Similar to the process in surface modification, the FIB-processed C-AFM tip was then used to carry, transfer and spray the MMP solutions onto the poly-Si wire sensor surface. Current flowing through the poly-Si wire I<sub>DS</sub> was then measured by using the semiconductor parameter analyzer Agilent 4156B.

### 3) FIELD EFFECT BIOSENSOR

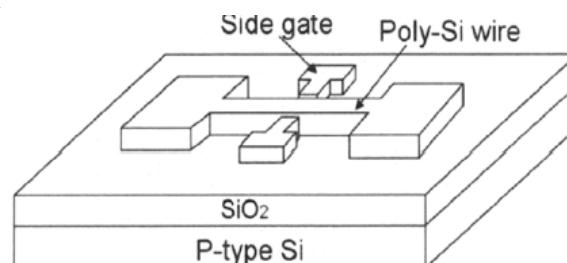


Fig .5 schematic diagram of polysilicon wire biosensor

[3]Fig 5 Here employing a simplified silicon chip fabrication technology to make ChemFET serve as biochemical sensors (biosensors) with low cost. We use a technique to coat analyte polymer/transducer on the top of gate dielectric film instead of gate metal of the conventional metal-oxide semiconductor field-effect transistor (MOSFET).

The biosensor is a field-effect transistor in which the metallic gate is omitted so that the dielectric material can be exposed to the analyte polymer directly from solution or via a transducer layer with a specific recognition function. The

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biosensor is a field-effect transistor in which the metallic gate is omitted so that the dielectric material can be exposed to the analyte polymer directly from solution or via a transducer layer with a specific recognition function. In the conventional MOSFET, the p-type silicon wafer as substrate forms the devices channel, whereas the dielectric film and the aluminium layer form the gate electrode, which are all protected by passivation films. In the ChemFET structure, the aluminium layer which covered the gate dielectric film is not required because the dielectric layer is responsible for the biochemical sensing. Therefore the passivation film is also completely removed in a region delimited by the area of the source and drain electrodes. To measure the current response of the detected materials, the terminal bias conditions are illustrated in Fig.6

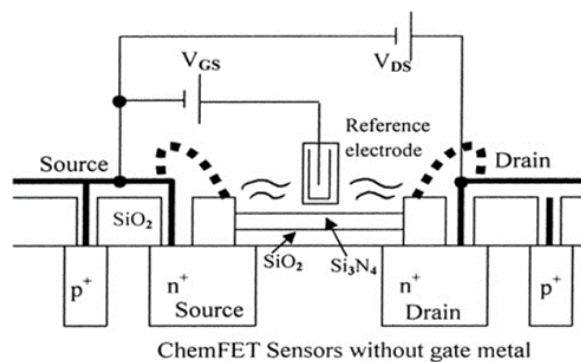


Fig.6 Schematic diagram showing the bias conditions used for testing the biosensors (ChemFET sensors) with Ag/AgCl reference electrodes

The biosensors and an Ag/AgCl reference microelectrode forming a three-terminal device are biased in the same way as a conventional MOSFET in the common source configuration, in which  $V_{GS}$  (V) is gate–source bias voltage and  $V_{DS}$  (V) is drain–source bias voltage. The output drain–source current,  $I_{DS}$  (mA), which indicates the current response for certain detected biomolecules and acids.

### III. COMPARATIVE STUDY

In first sensor, microfluidic chip sensor the resultant is shown in fig7. Graph shows the effect of flowrates on MCS response. After the glycerol sample injection into the inlet 2 flow stream, the sample traveled to the microfluidic device is hydrodynamically focused over the measuring junctions of the thermopile. The glycerol sample mixed with the water flowing from inlet 1 at the interface and generated heat. As the sample passed over the measuring junctions of the thermopile, the voltage increased and then returned to its baseline once the sample flowed past the thermopile. A typical response of the thermopile to glycerol water mixing is shown in Fig7a.

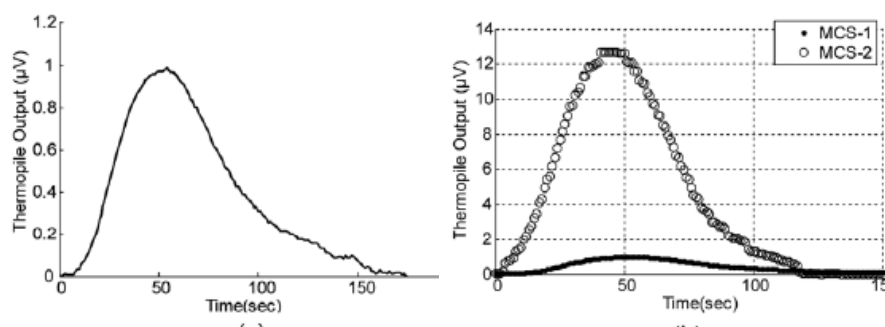


Fig. 7. (a) The output of MCS-1 for 10% (V/V) glycerol. Fig7 (b) Peak height comparison of MCS-1 and MCS-2 for 10 % (V/V) glycerol

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The response curve has two characteristics: 1) the magnitude, which represents the total temperature change detected by the thermopile due to the reaction, and 2) the area under the curve (AUC), this represents the total temperature heat detected by the thermopile. MCS-1 (thermopiles are outside of the flow channel) has high thermal resistance between thereaction zone and the thermopile compared to MCS-2 (thermopiles are inside of the flow channel) with only a 3  $\mu\text{m}$  photoresist layer. MCS-1 and MCS-2 responses to glycerol .Fig7 (b) Peak height comparison of MCS-1 and MCS-2 for 10 % (V/V) glycerolglycerol water mixing reaction were compared in Fig. 7b. MCS-2 showed improved performance compared to MCS-1. MCS-1 measured no voltage for small amount of sample glycerol concentration, because of the high thermal resistance for heat transfer to the thermopile. MCS-2 configuration showed 3.5-fold increase in sensitivity compared to MCS-1

The heat power sensitivity of the thermopile, the lowest heat power detected by MCS-2 was calculated to be 8.8 pW. Heat power sensitivity one of the achievement of MCS fabricated using a simple technique by integrating the thermopile sensor inside the flow channel. Major advantages of the MCSare sensing method is label-free:the principle is based on the calorimetric detection of heatreleased by the biochemical reactions, and MCS operateswithout power (self-generating): the sensor generates an outputwhen a temperature difference is maintained over its junctions.A voltmeter is only required to measure the output of thesensor. With the obtained heat power sensitives, the MCS canbe used for the detection of biochemical, and bioprocessesinvolving exothermic or endothermic reaction energies.A thermoelectric

microfluidic chip sensor developedto measure dynamic temperature changes in the orderof  $10^{-6}$  K. High sensitivity is obtained by an inexpensiveand easy to fabricate MCS without any extreme measuresand complex procedures to control reference temperatures.Two configurations of the sensors MCS1 and MCS-2. An improved sensor with low thermalresistance (MCS-2) showed a low heat power detection of8.8 pW. The sensitivity obtained with the simple to fabricateMCS is suitable for monitoring bioprocesses such as cellmetabolism, enzymatic reactions, and binding event measurements.

In the second sensor it is little expensive and have less sensitivity.Figure.8 shows the I-V characteristics of the poly-Si wire for solution with different glucose concentrations.The current  $I_{ds}$  flowing through the poly-Si wire increases with

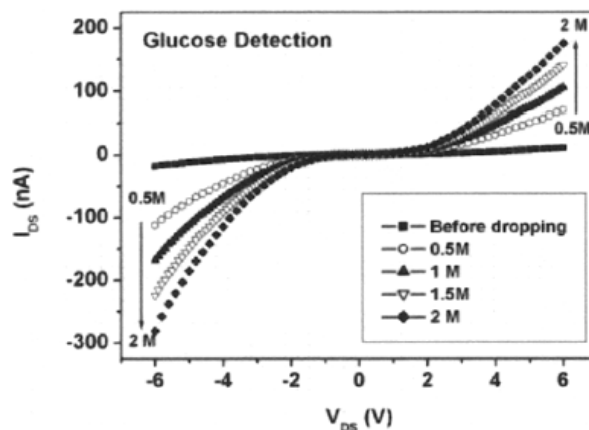


Fig 8. I-V characteristics

increasing glucose concentration for either polarity of the bias. One of the disadvantages is very less sensitivity because of we couldn't drop solution on exact position. It is possible only by using AFM tip.a poly-Si wire based biosensor for the detection of glucose solution and the MMP extracted from the humans lung adenocarcinoma cells A549 and H1299 with the help of an FIB processed C-AFM tip for the absorption, transferring as well as dropping of solution to be tested. By measuring the current flowing through the poly-Si wire, we successfully proved that the sensor can be used for biochemical sensing effectively.

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In the third one, it is highly expensive one because of the use of field effect transistor. First the repeatability and stability test for the biosensors, We selected two biosensor chips with the same sensitive areas of  $W/L = 20.0$  marked as chip 1# and chip 2#, respectively. Then, pure water and protein extracted from *Schistosoma cercariae* are used as detected materials. The biosensor's drain-source response currents for each detected material are measured 10–20 times, in order to verify the repeatability and stability performances of our fabricated biosensor. The process for our measurement is, firstly, coating the detected materials, such as, pure water or protein of *S. cercariae* on top of the sensor's gate dielectric film as the analyte polymer and then the sensor's drain-source current is measured.

As shown in Fig.9, it is clearly demonstrated that the detection experiment is not stable in its initial 1–10 times measurements however, after this initial period of time, the later detection experiment shows a relatively stable response current on our fabricated biosensor. Then, the detection experiments are conducted for series acids, for example, hexanoic acid, adipic acid and citric acid and so on. Also we can check and compare the biosensor's detection characteristics according to the carboxyl group and functional group of selected acids. The blank state measurement data are marked 'blank'. In acids case response current decreases with the increase of carboxyl group for serial alkyl-acid. Their current

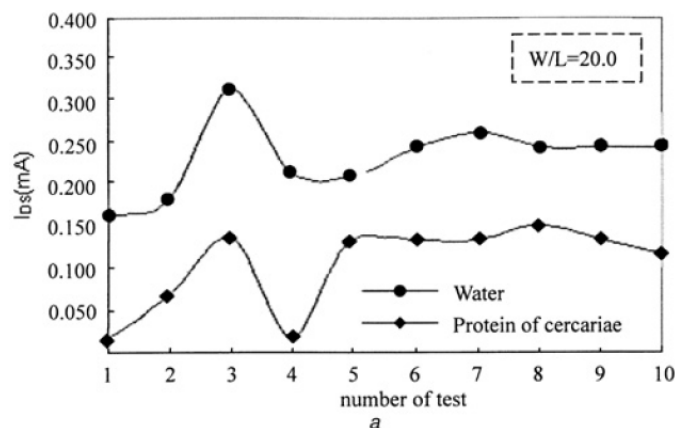


Fig 9 Repeatability and stability testing for our biosensors with detection materials of pure water and *S. cercariae* protein a Chip 1#

responses are smaller than the current response of the blank state. The biosensor's quantified sensitivity [12] is measured according to the series sensitive area and different concentrations of target detected biomolecules and acids. Haemocyanin, the protein extracted from snails, is used in this experiment.

The next detection experiment is conducted for *S. cercariae* [11] worms with different sensitive area, the measurement results are compared with that of pure water and blank, as shown in Fig.10. where (a) is for  $W/L = 4.20$  and (b) is for

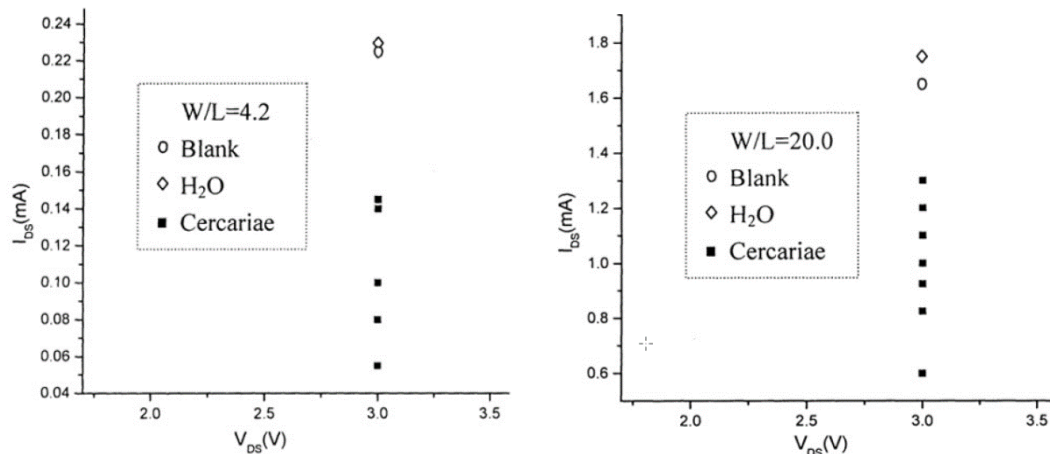


Fig 10 a. Current response distribution and comparison among the blank, pure water and *S. cercariae* worms with two chips a) W/L = 4.2, b) W/L = 20.0

W/L = 20.0. It can be seen that the response current for pure water and blank is relatively stable under certain experimental conditions. However, the response currents for *S. cercariae* worms are distributed in a certain current range. The reason for this data distribution range is (i) the variety of density for *S. cercariae* worms, (ii) the different positions on top of the sensitive area for each *S. cercariae* worms after finishing coating on the dielectric film, (iii) the characteristics of SAM *S. cercariae* worms and its formation conditions is related to each SAM processing. Since there are no or little electric dipole characteristics in pure water, the response current of pure water is positive and close to that of blank. However, *S. cercariae* worms have high dipole charge characteristics, properties of unique electronic conduction and delocalised electronic structure along the conjugated backbone, and its measured response currents are negative and far apart from that of pure water and blank.

From these comparative study polySI wire sensor is less sensitive and it is applicable only for specified samples. the sensors external voltage. External power is required for the working of FET sensor also, it is highly expensive. The biosensor depending on number of repeatable tests for getting proper result, so sensitivity is very less. by comparatively Microfluidic chip sensor which is highly efficient, inexpensive, high sensitive sensor.

#### IV. CONCLUSION

From the above analysis and study of three different sensors used for bio chemical detection, by comparing these sensors, we can conclude that the microfluidic chip sensor is most efficient, sensitive, and have stability also. The other two are less sensitive and require the use of power generator also. Two major advantages of the MCS developed in this study, One is the sensing method is label-free: the principle is based on the calorimetric detection of heat released by the biochemical reactions, and second is MCS operates without power (self-generating): the sensor generates an output when a temperature difference is maintained over its junctions. The other two are less sensitive and require the use of power generator also. The poly-Si wire based biosensor is used only for the detection of glucose solution and the MMP extracted from the humans lung adenocarcinoma cells. The main limitation is that, it is used for some specific samples. Field effect biosensor is highly expensive.

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